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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/203,078
Filing Date: December 01, 1998
Appellant(s): ZHANG ET AL.

Gina N. Shishima
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed July 25, 2005 appealing from the Office action mailed November 17, 2004.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is incorrect. A correct statement of the status of the claims is as follows:

This appeal involves claims 1-31 and 38-62.

Claim 31 has been canceled.

Claims 33-37 are withdrawn from consideration as not directed to the elected subject matter.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

GROUNDS OF REJECTION NOT ON REVIEW

The following grounds of rejection have not been withdrawn by the examiner, but they are not under review on appeal because they have not been presented for review in the

appellant's brief. Provisional Double Patenting Rejection between instant claims 1 and 29 and claims 30 and 31 of 09/880,609.

WITHDRAWN REJECTIONS

The following grounds of rejection are not presented for review on appeal because they have been withdrawn by the examiner.

Claims 1, 3-9, 13-28, 30, 31, 38-49 and 51-62, rejected under 35 U.S.C. 102(a) as being anticipated by Zhang et al. (WO 98/22588), as further evidenced by Wu et al. (US 6,689,600 B1).

Claims 1, 3-9, 13-28, 30, 31, 38-49 and 51-62, rejected under 35 U.S.C. 102(e) as being anticipated by Zhang et al. (US Patent No. 6,194,191 B1), as further evidenced by Wu et al. (US 6,689,600 B1).

Claims 10-12 and 29, rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al. (WO 98/22588) **or** Zhang et al. US (6,194,191) as applied to claims 1, 3-9, 13-28, 30, 31, 38-49 and 51-62.

Claims 2 and 50, rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al. (WO 98/22588) **or** Zhang et al. US (6,194,191) as applied to claims 1, 3-31, 38-49 and 51-62 and further in view of Graham et al. (C31 of IDS) and Leu et al. (6,194,210 B1).

NEW GROUND(S) OF OBJECTION

These grounds of objection were presented in the Office action of October 3, 2003 and are reinstated since the teachings of Zhang et al. (WO 98/22588) **or** Zhang et al. US (6,194,191) are not applicable against the instant claims.

Claims 5-7 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. The prior art does not teach or suggest perfusion rates to maintain a low concentration of glucose.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

HUYGHE B.G. et al. "Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 by Column Chromatography" Human Gene Therapy, vol. 6, pages 1403-1416.

KUCHLER R.J. *In* Biochemical Methods in Cell Culture and Virology. Stroudsburg, Penn: Dowden, Hutchingson & Ross, Inc., 90, 91, 99, 100, 1977.

6,194,210 B1 LEU et al. 2-2001

GRAHAM et al. *In* Methods in Molecular Biology: Gene Transfer and Expression Protocols 7. Murray, E.J. Editors. Clifton, NJ: Humana Pres, 109-120 and 205-225, 1991.

GARNIER et al. "Scale-up of the adenovirus expression system for the production of recombinant protein in human 293S cells" Cytotechnology, vol 15, 1994, pages 145-155.

SPIER, R.E. and J.B. Griffiths, eds., *In* Animal Cell Biotechnology, vol. 3, pages 179-219, 1988.

MEDIATECH TECHICAL INFORMATION BULLETIN (No citation information available - provided by Appellant as an attachment to an affidavit submitted February 28, 2002.)

MURPHY et al. Virus Taxonomy . *In* B.N. Fields et al. (ed.), Fields Virology, 3rd ed. Philadelphia: Lippencott-Raven Publishers; 1996: Table 6, pages 51-54.

5,106,841 SCHEER 4-1992

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3, 8, 9, 13-25, 31, 38, 47, 49 and 51-62 are rejected under 35 U.S.C. 102(b) as being anticipated by Huyghe et al. (Human Gene Therapy. 1995; 6: 1403-1416) in light of Kutchler et al. *In* Biochemical Methods in Cell Culture and Virology. Stroudsburg, Penn: Dowden, Hutchingson & Ross, Inc., 90, 91, 99, 100, 1977 (supplied by Appellant February 28, 2002 as Exhibit 5).

The claims are drawn to a process of preparing adenovirus by preparing a culture of producer cells essentially homogenous with respect to growth, infecting the producer cells with adenovirus between mid-log and stationary phase, and harvesting the adenovirus and placing the virus into a pharmaceutically acceptable composition. The cells are allowed to attach to a surface between 3 and 24 hours prior to infection and are recirculated during infection. The adenovirus is replication-defective, lacking a portion of E1, which is complemented by 293 cells, and encodes the p53 gene from a CMV promoter. The adenovirus is purified by only one or several chromatographic separations including ion-exchange chromatography.

Huyghe et al. anticipate preparing adenovirus by preparing a culture of 293 producer cells that have a attained an essentially homogenous confluency of 50-60% when the cells are infected

with a replication-defective adenovirus expressing p53 from a CMV promoter in place of E1 coding sequences. This percentage of confluency reasonably corresponds to mid-log phase of cell growth (explained in greater detail below). The 293 cells are allowed to attach to the surface between 2 and 2.5 days prior to infection and upon infection, the virus was mixed thoroughly with the cell culture medium. The adenovirus is harvested and added to phosphate-buffered saline supplemented with 2% sucrose and 2 mM $MgCl_2$, a pharmaceutically acceptable carrier. The adenovirus of Huyghe et al. is purified by several methods of chromatography, including ion-exchange chromatography. See the first full paragraph of the second column on page 1403, "Production of infected ATCC 293 cells", "Harvest and lysis", "Preparation of ACN53 standard material" and "Chromatographic parameters" bridging pages 1404-1405.

When the teachings of Kuchler et al. (supplied by Appellant February 28, 2002 as Exhibit 5) are applied to the cells of Huyghe et al., Appellant concluded (in the submission on March 8, 2004) that the cells of Huyghe et al. are barely out of lag phase since the lag times of 293 cells ranges between 24-48 hours. However, the Examiner disagrees since Huyghe et al. infected the cells between 48 and 60 hours (i.e. between 2 and 2.5 days) after seeding, see the second paragraph under "Production of infected ATCC 293 cells" on page 1404. Therefore, the supported facts provided by the references and the declaration are:

1) the lag time of 293 cells ranges between 24-48 hours

2) the cells of Huyghe et al. are have a confluency of 50-60% upon infection

3) the cells of Huyghe et al. attach to the surface of the plate for 48 to 60 hours before infection, which is beyond the hours required for the lag phase

4) the chart provided by Kuchler indicates that the growth curve of cells after 60 hours of incubation is the mid-point of the growth curve, i.e. mid-phase.

Therefore, from the factual evidence available, it is determined that the cells of Huyghe et al. are at mid-phase upon infection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 10-12 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huyghe et al. as applied to claims 1, 3, 8, 9, 13-25, 31, 38, 47, 49 and 51-62 above.

The claims are drawn to specific seeding densities of the producer cells and particular characteristics of the harvested adenovirus.

Although Huyghe et al. do not teach the specific cell numbers to be plated, the number would be a subjective determination by one of ordinary skill based on many factors, such as the type of cell, the condition of the cells before plating, and the nature of the cell's division, ect. Therefore, it would be prima facie obvious for one skilled in the art to determine the appropriate number of cells to plate for each situation encountered.

Further, although none of the references, in the alternative, teach a harvested adenovirus with the characteristics listed in claim 29, all of the references teach various methods of improving the quantity and/or purity of the recombinant virus obtained. Therefore, it would have

been prima facie obvious to one of ordinary skill to test any one of the properties listed to ensure a good yield of adenovirus.

Claims 2 and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huyghe et al. as applied to claims 1, 3, 8, 9-12, 13-25, 29, 31, 38, 47, 49 and 51-62 above and further in view of Graham et al. (C31 of IDS) and Leu et al. (6,194,210 B1).

Claims 2 and 50 require that the cells are infected in late-log and stationary phase of growth.

See the teachings of Huyghe et al. Huyghe et al. do not teach infecting at late-log to stationary phase of cell growth.

Leu et al. teach a method of producing large quantities of virus by allowing uniform attachment of cells, growing the cells to late-log phase with medium replenishment to provide adequate cell nutrition and infecting the cells at late-log phase and harvesting the virus, see column 11, lines 18-column 12, line 9 and claims 1 and 4. One of ordinary skill in the art at the time the invention as made would have been motivated to have propagated the adenovirus of Huyghe et al. with the cell culture method steps of infection of Leu et al. to increase the amount of adenovirus produced in cell culture. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of growing the adenovirus of Huyghe et al. with the cell culture method steps taught by Leu et al. because Leu et al. teach that a wide range of viruses may be propagated to generate vaccines using the method steps, see column 5, lines 29-31. Therefore, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art, absent unexpected results to the contrary.

Leu et al. specifically teach infecting at late log phase and provide a clear motivation, i.e., to produce large quantities of virus, see claim 1 for example. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success for combining the teachings of Leu et al. with Huyghe et al. not only because the method of Leu et al. is applicable to general viral propagation, but also because Huyghe et al. teach infection of adenovirus at least at mid-log phase. Further, Mediatech's Technical Information demonstrate that cells of at least 70% confluency are in log-phase. Therefore, cell confluency of 80-90% at the time of infection would certainly be at late-log phase. Graham et al. (reference C31 of the IDS submitted September 13, 2001) teaches infecting cells at 80-90% confluency with adenovirus, see section 3.1.2 on page 117. Graham et al. clearly demonstrate that the teachings of Leu et al. are applicable to adenovirus infection in cells at late-log phase of growth. Therefore, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art, absent unexpected results to the contrary.

Claims 26-28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huyghe et al. as applied to claims 1, 3, 8, 9-12, 13-25, 29, 31, 38, 47, 49 and 51-62 above, and further in view of Graham et al. (C7) for reasons of record.

The claims are drawn to lysing the producer cells with a detergent by means other than freeze-thaw. Graham et al teach that 5% sodium deoxycholate can be used to disrupt cells without disrupting adenovirus virions, see page 119. Therefore it would have been obvious to use deoxycholate or another detergent as an alternative method to lyse the infected cells. Further, autolysis would be a conventional alternative to detergent lysis.

Claims 4, 30, 39-46 and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huyghe et al. as applied to claims 1, 3, 8, 9-12, 13-25, 29, 31, 38, 47, 49 and 51-62 above, and further in view of Garnier et al. (C26) and Spier et al. (C35 of the IDS) .

The claims require the producer cells to be perfused for a portion of the time the cells are cultured. The claims also require the cells to be cultured in various systems including a microcarrier, multiplate, perfused packbed reactor, microencapsulation or bioreactors, such as stirred tank, airlift or sparge.

Huyghe et al. do not teach perfusion or the various culture systems recited.

However, Garnier et al. teach scale-up adenovirus growth using medium replacement for controlling glucose concentrations for improved virus yields in a bioreactor, see the material and methods section. One of ordinary skill in the art at the time the invention was made would have been motivated to used the system of Garnier in the method of Huyghe et al. to produce larger quantities of adenovirus. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success in combining the teachings of Garnier et al. and Huyghe et al. because both references culture 293 cells for the propagation of adenovirus.

Neither Huyghe et al. nor Garnier et al. teach the various culture systems claimed. However, Spier et al. review each of the various culture systems claimed, see the entire reference. One of ordinary skill in the art at the time the invention was made would have been motivated to use a conventionally applied culture system, described by Spier et al. in the method and system of Huyghe et al. and Garnier et al. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success in using any of the culture systems of Spier et al. in the method of Garnier et al. and Huyghe et al. because Garnier

et al. use a bioreactor system to propagate large quantities of adenovirus and Spier et al. review various types of conventional bioreactor systems. Therefore, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art, absent unexpected results to the contrary.

(10) Response to Argument

Appellant asserts on page 7 that the Examiner has not shown that the characteristics of the invention are present in the prior art and has not provided a basis to reasonably support the determination. More particularly, Appellant argues that inherent anticipation is not shown because the Examiner relies on data from dissimilar cell types and fails to connect the data to the cells of the present invention.

At the bottom of page 7 of the Appeal to page 9, Appellant reviews some of the correspondence history. Appellant irrelevantly mentions the Office Action of September 13, 2001, in which the Office inadvertently confused cell cycle phases with cell growth phases. As stated in the Office Action mailed October 3, 2003, the rejections applied on September 13, 2001 are no longer applicable.

Appellant continues by summarizing the conclusions of the Declaration submitted under 37 CFR § 1.132 by Shawn Gallagher. In this Declaration, Mr. Gallagher attempts to explain how it might be possible to estimate the phase of Huyghe's cells at the time of infection with admitted assumptions and extrapolations, see pages 6 and 7 of the Declaration and the last two paragraphs on page 8 and the first paragraph on page 9 of the Appeal Brief.

In the Declaration, Mr. Gallagher assigns an arbitrary number for Huyghe's

seeding cell density and further assumes the amount of time the 293 cells of Huyghe are in lag time. Mr. Gallagher then applies this arbitrary number and assumed amount of time in a calculation to arrive at a number representing a concentration of cells consistent with early log phase density, see page 7 of the Declaration. This discussion provided by Mr. Gallagher was found to be speculative and unsubstantiated since the data used to arrive at the conclusion is admittedly assumed. Both the Office and Appellant agree that Huyghe et al. do not discuss seeding densities. However, the Office does not and has not ventured to speculate or assume seeding densities that cannot be substantiated by Huyghe et al., as Appellant has, since the reference does not discuss seeding densities. Appellant has not provided any argument or data sufficient to support the assumptions present in the declaration.

Appellant further reviews the references submitted to the Office with Mr. Gallagher's Declaration, MediaTech's Technical Information Bulletin and the teachings of Kuchler at the bottom of page 9 of the Appeal. With respect to the teachings of Mediatech's Technical Information, Appellant equates 70% confluency with log phase and reasons that 50%-60% confluency is only in early log phase at the very most.

In response, the Office stated that Mediatech correlates cells that are at least 70% confluent with log phase, see the top of the second column:

"Subculturing is usually performed during the log phase....[c]heck for cultures that appear to be at least 70% confluent."

However, Mediatech does not differentiate cell confluency with the degree of log phase the cells may be in. For instance, Mediatech does not teach whether 70% confluency correlates to early, mid or late log phase. The reference only provides a general teaching that 70%

confluency is log phase. Without any guidance provided by Mediatech regarding the percentage of confluency and the stage of log growth, it is maintained that a definite identification of which log phase a cell culture is in based on a percentage of confluency cannot be determined.

Appellant, citing the teachings of Kuchler, determines that the cells of Huyghe et al. are barely out of lag phase since the lag times of 293 cells ranges between 24-48 hours and Huyghe et al. infected the cells between 48 and 60 hours after seeding.

In view of the information provided by Kuchler regarding the lag times of 293 cells in view of the information provided by Huyghe about the cells and infection, the following supported facts are listed:

1) the lag time of 293 cells ranges between 24-48 hours

2) the cells of Huyghe et al. are have a confluency of 50-60% upon infection

3) the cells of Huyghe et al. attach to the surface of the plate for 48 to 60 hours (i.e. 2 to 2.5 days, see second paragraph under "Production of infected ATCC 293 cells on page 14.4) before infection, which is beyond the hours required for the lag phase according to the teachings of Kuchler, provided by Appellant.

4) the chart provided by Kuchler indicates that the growth curve of cells at 60 hours of incubation is the mid-point of the growth curve, i.e. mid-phase. To more clearly show this, the Examiner drew a straight vertical line, parallel to the Y-axis, from the 60 hour mark on the X-axis through the growth curve of Figure 3-1 on page 91 of Kuchler and supplied Appellant with a copy of the drawing. The point at which the drawn line meets the growth curve is clearly at the mid-point of the growth curve.

Therefore, from the factual evidence available from the teachings of Huyghe and the teachings of Kuchler, provided by Appellant, it is maintained that the cells of Huyghe et al. are at mid-phase upon infection.

Appellant argues that the Examiner cannot reasonably support a determination that Huyghe infected the 293 cells at mid-log phase since there is no nexus between the fibroblasts of Kuchler grown in suspension and the monolayered 293 cells of Huyghe.

Appellant's arguments have been fully considered, but are found unpersuasive. Appellant supplied the Kuchler reference to the Office as Exhibit 5 of the amendment submitted February 28, 2002 to rebut the teachings of Huyghe. On page 11 of the February 28, 2002 response and paragraph 10 of Mr. Gallagher's Declaration, it is argued that the lag phase period of 24 to 48 hours, discussed by Kuchler, applies to the cells of Huyghe. Therefore, Appellant established the nexus between the cells of Kuchler and the cells of Huyghe. The Office has applied the same nexus between the cells of Kuchler and Huyghe, established by Appellant, with respect to the log phase of cell growth. However, since the Office determined that this teaching in Kuchler is also relevant to the teachings of Huyghe, Appellant disputes a connection between the cells. It remains unclear how one aspect of Kuchler is applicable to the cells of Huyghe while another aspect is not.

Appellant argues alternatively, that even if Kuchler were relevant to the teachings of Huyghe, the growth curve shows the cells to be slightly before the mid-log phase at 60 hours.

In response, the chart provided by Kuchler indicates that the growth curve of cells at 60 hours of incubation is the mid-point of the growth curve, i.e. mid-phase. To more clearly show this, the Examiner drew a straight vertical line, parallel to the Y-axis, from the 60 hour mark on

the X-axis through the growth curve of Figure 3-1 on page 91 of Kuchler and supplied Appellant with a copy of the drawing. The point at which the drawn line meets the growth curve is clearly at the half-way or mid-point of the growth curve.

Appellant also argues that the chart does not provide evidence that the cells of Huyghe were infected after mid-log phase of growth (emphasis added). However, claim 1 only requires that the cells be infected between mid-log phase and stationary phase. Mid-log would be the lower end of the range recited and Huyghe infect cells at mid-log, as evidenced by the teachings of Kuchler.

Appellant also states that from the chart, the fibroblasts of Kuchler have a doubling time of 16 to 20 hours, which is quicker than the 36 hours of doubling time assumed by Mr. Gallagher.

However, the calculations shown by Mr. Gallagher were derived from admittedly assumed numbers and do not offer conclusive evidence.

Appellant notes that the doubling time derived from Mr. Gallagher is consistent with the data provided by the Quality Assurance Report, which shows a doubling time of approximately 30 hours for 293 cells, presented with the Declaration of Dr. Zhang as Exhibit 8 on September 3, 2004. Appellant concludes that a doubling time of 30 hours is well below mid-log according to Figure 1-3 of Kuchler.

However, while the Quality Assurance Report states that the cell doubling time is approximately 30 hours, the Report does not indicate or imply which stage of log phase, i.e. early, mid or late log, the cells are in during this exponential growth phase. Further, the cells of

Huyghe were infected 2 to 2.5 days after seeding, i.e. between 48 and 60 hours, not 30. According to Kuchler, 60 hours equates to mid-log.

With regard to seeding densities in section 4 of the Brief, Appellant concludes that the burden has not been properly shifted to Appellants to provide substantiated evidence of Huyghe's seeding density.

In response, Appellant appears to have strayed from the issues since seeding density is not a recited element in any of the claims anticipated by Huyghe as evidenced by Kuchler. In addition, the discussion of seeding density by Appellant is taken out of context. In the Declaration by Shawn Gallagher submitted February 28, 2002, Appellant arbitrarily assumed numbers for the seeding densities of Huyghe et al. for a calculation. The Office does not and has not ventured to speculate or assume seeding densities that cannot be substantiated by Huyghe et al., as Appellant has, since the reference does not even discuss seeding densities. The Office is not required to find support for assumptions made by Appellant about seeding densities. Seeding density numbers are not recited in the claims anticipated by Huyghe. Therefore, arguments drawn to seeding density are irrelevant since both Appellant and the Office agree that Huyghe do not teach seeding densities.

Appellant states that the Offices mischaracterizes the teachings provided in Mediatech's Technical Information. Appellant argues that cultures that are less than 70% confluent cannot be assured of even being in log phase in view of the information provided by Mediatech.

A review of the reference has been considered, but is found unpersuasive. At the top of the second column of Mediatech's Technical Information it states:

"Subculturing is usually performed during the log phase....[c]heck for cultures that appear to be at least 70% confluent."

This teaching does not differentiate cell confluency with the different stages of log phase the cells may be in. For instance, Mediatech does not teach whether 70% confluency correlates to early, mid or late log phase. The reference only provides a general teaching that 70% confluency is log phase. Without any guidance provided by Mediatech regarding the percentage of confluency and the stage of log growth, it is maintained that a definite identification of which log phase a cell culture is in based on a percentage of confluency cannot be determined.

Appellant concludes that since the Examiner has not established a nexus between the cells of Kuchler and Huyghe and "insists that without crucial knowledge of seeding density any determination of whether the cells of Huyghe were in log phase at the time of infection would be speculative and unsubstantiated", the Examiner cannot establish mid-log phase of infection by Huyghe.

In response, Appellant's conclusions are incorrect. The Office does not need to establish a nexus between the cells of Huyghe and Kuchler because Appellant established this connection in Exhibit 5 of the amendment submitted February 28, 2002 to rebut the teachings of Huyghe. In addition, it is unclear where any "insisting" regarding seeding density takes place in any correspondence from the Office. As discussed above, Mr. Gallagher's Declaration introduced arbitrary seeding density numbers. Along with this Declaration, Appellant provided a Freshney reference and Mediatech's Technical Information reference. In each of these references, it is taught that seeding density is established in the art as a crucial component of the log phase, see "The Log Phase" of Freshney on page 239 and "Growth Phases" in Mediatech Technical

Information. However, since Huyghe does not provide any information regarding the initial density of cells, Appellant's conclusion of early log phase density obtained by a calculation using assumed and arbitrary values for the cells of Huyghe et al. is speculative and unsubstantiated.

The only data Huyghe supplies is that 293 cells are seeded and that the cells reach 50-60% confluency between 2 (48 hours) to 2.5 (60 hours) days after seeding. Any inferences with respect to seeding densities or any other aspect not taught by Huyghe is unsubstantiated. The length of time between seeding and infection and the confluency level at infection are all there is to go on to determine mid-log phase. The teachings of Kuchler, provided by Appellant as relevant to the cells of Huyghe, correlates the amount of time in Huyghe with mid-log by the cell growth curve in Figure 3-1. Therefore, it is maintained that Huyghe anticipate the invention claimed as evidenced by Kuchler.

Appellant further asserts that dependent claims are also not anticipated by Huyghe in view of Kuchler. With respect to claim 3, Appellant argues that the Examiner has not pointed out any mention of "homogeneous" producer cells mentioned in Huyghe.

Contrary to Appellant's assertions, the Office has specifically taught where this limitation is found, which is summarized in Appellant's discussion on page 15. In the second paragraph of the Materials and Methods section on page 1404, Huyghe specifically state that the cells were infected when they reached about 50-60% confluency. This teaching meets the limitation of claim 3, requiring that the cells "are essentially homogeneous with respect to the phase of growth".

Appellant argues that all of the limitations of claim 8 have not been met.

In response, the cells of Huyghe are allowed to attach for 48 to 60 hours prior to infection. This range encompasses the 3 to 24 hours of attachment required by the claims. The claims do not require, as Appellant implies, that the period for attachment ends at hour 24 or that infection occurs at hour 24. The claim merely requires between 3 and 24 hours of attachment prior to be infected. The infection by Huyghe at between hours 48 and 60 meets this limitation.

Appellant argues that Huyghe provides no indication that the media was recirculated during the infection step.

A review of Huyghe has been fully considered, but is found unpersuasive. Huyghe explicitly teaches adding virus to cell culture medium and mixing, i.e. recirculating, thoroughly, see the second paragraph under the Materials and Methods section on page 1404.

Appellant argues that is has not bee shown that the harvested adenovirus of Huyghe is purified and placed in a pharmaceutically acceptable carrier.

Huyghe specifically teaches harvesting the virus, i.e. purifying it from all cell debris, see "Harvest and lysis" on page 1404. Additionally, Huyghe teach that the harvested virus is added to phosphate-buffered saline supplemented with 2% sucrose and 2 mM $MgCl_2$, see "Preparation of ACN53 standard material" and "Chromatographic parameters" bridging pages 1404-1405. Phosphate-buffered saline is a conventional pharmaceutically acceptable, see column 4, lines 61-65 of US 4,987,141 for example.

With respect to claim 31 and 58, Appellant argues that Huyghe does not anticipate these claims for reasons provided above. However, none of the arguments have been found persuasive and the rejections of these claims are maintained.

In section D, starting on page 21 of the Brief, Appellant argues that claims 10-12 are not rendered obvious by the teachings of Huyghe. More specifically, Appellant argues that Huyghe does not provide motivation or suggestion to one of ordinary skill in the art that would determine seeding density to infect adenovirus between mid-log and stationary growth or a reasonable expectation of success since seeding density is an admittedly crucial criterion by the Examiner.

Appellant's arguments have been fully considered, but are found unpersuasive. It is established by the teachings of the Kuchler that Huyghe et al. infect the cells at mid-log phase. Huyghe et al. do not disclose subjective factors, such as the condition of the cells before plating or the nature of the cell divisions, ect., but since the cells are required by the reference to be "about 50-60%" confluent at the time of infection, the exact number of cells plated by Huyghe et al. would have been a critical element in order to reach the required log phase by 60 hours. Since it is established from Kuchler that Huyghe infect cells at mid-log phase and since culturing cells for the purpose of propagating virus has been practiced since the 1950's, it is maintained that plating density would be knowledge generally available to one of ordinary skill in the art at the time the invention was made.

With respect to claim 29, Appellant argues that claim 29 is not directed to testing adenovirus, but is drawn to adenovirus preparations that meet these limitations.

Claim 29 and the teachings of Huyghe in view of Kuchler have been reviewed. Huyghe does not teach any of the properties listed. However, Huyghe et al. teach a method of improving the quantity and/or purity of the recombinant virus obtained. Since improving quantity is quantity is obviously a motivation for the ordinary artisan in the adenovirus art, it would have

been prima facie obvious to one of ordinary skill to quantify any one of the properties listed to ensure a good yield of adenovirus.

In section G on page 24, Appellant argues that claims 2 and 50 are not rendered obvious by Huyghe in view of Graham and Leu. More specifically, Appellant argues that the ordinary artisan would not be motivated to incorporate the teachings of Leu into the adenovirus production method of Huyghe. Appellant further argues that there is no reasonable expectation to combine the references since Leu teaches producing hepatitis A in MRC-5 cells and Huyghe teaches producing adenovirus in 293 cells. Appellant argues that there is no mention of adenovirus in the entire method of Leu and that none of the viruses mentioned belong to the adenovirus family. Appellant then discusses the differences between adenoviruses and hepatitis A. Appellant additionally point out that there is no mention of 293 cells used in the method of Leu and asserts that the Examiner fails to explain how the mention of any cell type Leu mentions is related to what Huyghe teaches.

Appellant's arguments and a review of the references have been fully considered, but are found unpersuasive. The method of viral propagation taught by Leu et al. is clearly applicable to a wide range of unrelated virus families. In column 5, lines 29-32, Leu specifically state that viruses " are not limited to" those named. Leu lists a wide range of viruses that are applicable for use with the method. These include hepatitis A, rubella and polioviruses, which are positive, single stranded RNA viruses, measles, mumps and rubella, which are negative, single stranded RNA viruses, Rotavirus, a double stranded RNA viruses, varicella and herpes viruses, which are double stranded DNA viruses. Adenovirus is also a double stranded DNA virus. See MURPHY et al. Virus Taxonomy . *In* B.N. Fields et al. (ed.), Fields Virology, 3rd ed. Philadelphia:

Lippencott-Raven Publishers; 1996: Table 6, pages 51-54. Therefore, the teachings of Leu et al. are clearly a teaching applicable to the general viral propagation art, which includes adenoviruses.

Additionally, Leu teach that the method can be used to propagate virus in various host cells. Leu lists some conventionally used cells in column 5, lines 29-31. While Leu focuses discussions on MRC-5 cells, as Appellant states, the reference does not teach that this is a conventional cell type for adenovirus production. However, as evidenced by the teachings of Scheer (US 5,106,841), it is clearly evident that adenovirus is conventionally propagated in MRC-5 cells, see Tables 1 and 2 in column 11. Therefore, it is maintained that the teachings of Leu are applicable to the adenovirus art.

Appellant further asserts that there is no teaching from Leu that would indicate increased hepatitis A production. However, claim 1 of Leu is drawn to a method of producing "large quantities" of virus.

Appellant also states that there is no indication from Huyghe that there is a problem that needs to be solved with regard to increased virus production or why the ordinary artisan would turn to the teachings of Leu for virus production.

Appellant's argument has been fully considered, but is found unpersuasive since the amount of virus produced is a general motivation relevant to any ordinary artisan in the virus propagation arts, as evidenced by the teachings of Leu.

Leu et al. specifically teach infecting at late log phase and provide a clear motivation, i.e., to produce large quantities of virus. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success for combining the teachings of Leu et

al. with Huyghe et al. not only because the method of Leu et al. is applicable to general viral propagation, but also because the primary references teach infection of adenovirus at least at mid-log phase. Further, Mediatech's Technical Information demonstrate that cells of at least 70% confluency are in log-phase. Therefore, cell confluency of 80-90% at the time of infection would certainly be at late-log phase. Graham et al. (reference C31 of the IDS) teaches infecting cells at 80-90% confluency with adenovirus, see section 3.1.2 on page 117. Graham et al. clearly demonstrate that the teachings of Leu et al. are applicable to adenovirus infection in cells at late-log phase of growth. Appellant doe not argue that Graham does not teach infecting at late-log phase. Therefore, it is maintained that the invention is prima facie obvious in view of the combination of teachings cited.

In section I, Appellant asserts that claims 26-28 are not rendered obvious because there is no motivation to combine the teachings of Huyghe and Graham.

Appellant's arguments have been fully considered, but are found unpersuasive. Graham et al teach that 5% sodium deoxycholate can be used to disrupt cells without disrupting adenovirus virions, see page 119. Therefore it is maintained that it would have been obvious to use deoxycholate or another detergent as an alternative method to lyse the infected cells. Further, autolysis would be a conventional alternative to detergent lysis.

In section J, Appellant argues that claims 4, 30, 39-46 and 48 are not rendered obvious by Huyghe, Garnier and Spier because none of the references teach infection at mid-log phase.

However, this assertion is not persuasive in view of the teachings of Huyghe as evidenced by Kuchler. The cells of Huyghe et al. are have a confluency of 50-60% upon infection and are infected 48 to 60 hours after plating. The chart provided by Kuchler indicates

that the growth curve of cells after 60 hours of incubation is the mid-point of the growth curve, i.e. mid-phase. Therefore, from the factual evidence available, it is determined that the cells of Huyghe et al. are at mid-phase upon infection. Neither Spier nor Garnier are required to teach that which is taught by Huyghe as evidenced by Kuchler.

Appellant argues that there is no motivation to combine the goals of Garnier with the goals of the present application because Garnier only concerns the production of heterologous proteins and does not concern the production of adenovirus. Appellant argues that there is no indication that an increase in protein necessarily implies an increase in virus yield. Appellant concludes that the reference teaches away from increased virus production at the expense of making more protein.

Appellant's arguments have been fully considered, but are found unpersuasive. The scale-up method of Garnier is to improve the volumetric yield of the [adenovirus] recombinant protein production system, see the introduction section. This teaching necessarily requires an increased yield of adenoviruses carrying the heterologous protein. An increase of protein produced/or expressed by the adenovirus necessarily means that more adenovirus is present in greater quantities, i.e. a volumetric yield. In normal and recombinant processes utilizing tissue culture, viral nucleic acids are transcribed and translated into proteins, which assemble into virus particles. That is, the more protein that is produced, the more virus particles are realized. The heterologous protein measured by Garnier is produced by recombinant adenovirus expressing the protein. Furthermore, Garnier et al. specifically state that one of the goals of the method is to increase "AV stocks" (adenovirus), see the abstract and the first paragraph of the introduction.

Finally, Appellant argues that Spier does not mention adenoviruses or any bioreactor system that might support their propagation.

A review of Spier has been considered, but is found unpersuasive. Garnier use a bioreactor system to propagate adenovirus, see pages 152-153. Spier review the conventional types of bioreactor systems conventionally used in the virus propagation art. Therefore, it is maintained that one of ordinary skill would have been motivated to use a conventionally applied culture system, described by Spier et al. in the method and system of Huyghe et al. and Garnier et al. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success in using any of the culture systems of Spier et al. in the method of Garnier et al. and Huyghe et al. because Garnier et al. use a bioreactor system to propagate large quantities of adenovirus and Spier et al. review various types of bioreactor systems.

Therefore, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art, absent unexpected results to the contrary.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Sharon A. Foley

Conferees:

James Housel 10/17/05

Christina Chan 10/17/05